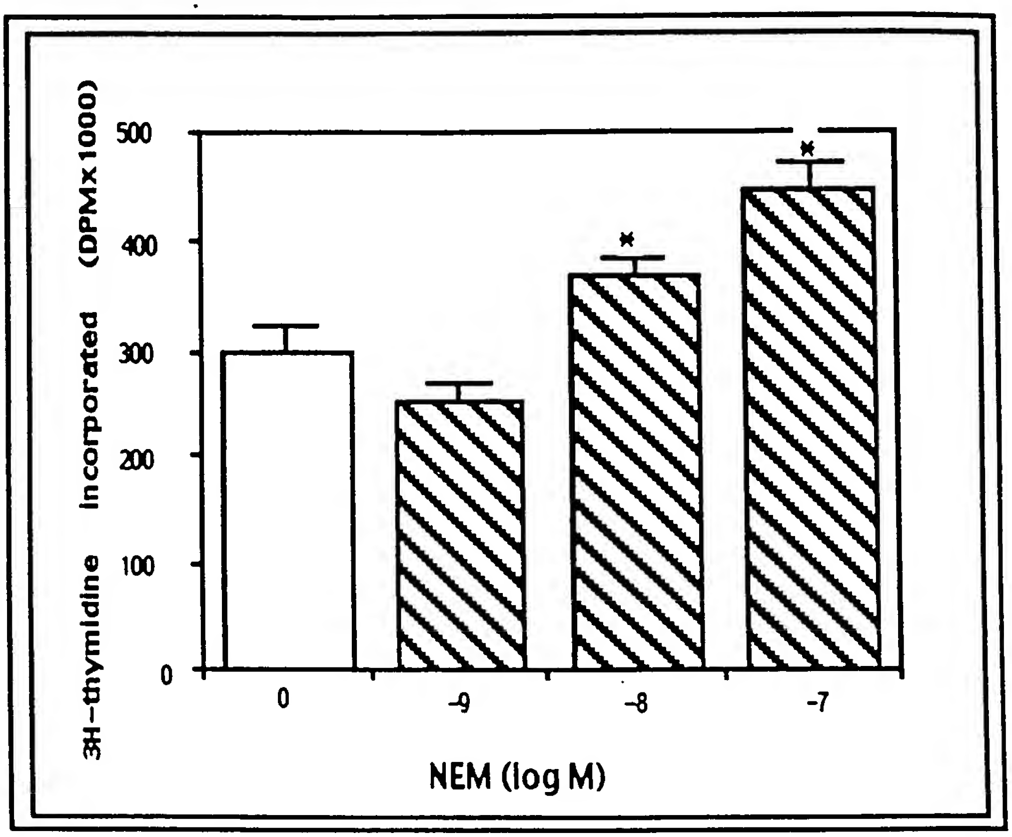

3H-thymidine Incorporated (DPMx1000)
500
400
300
200
100
0
0
-9
-8
-7
NEM (log M)

FIG. 1

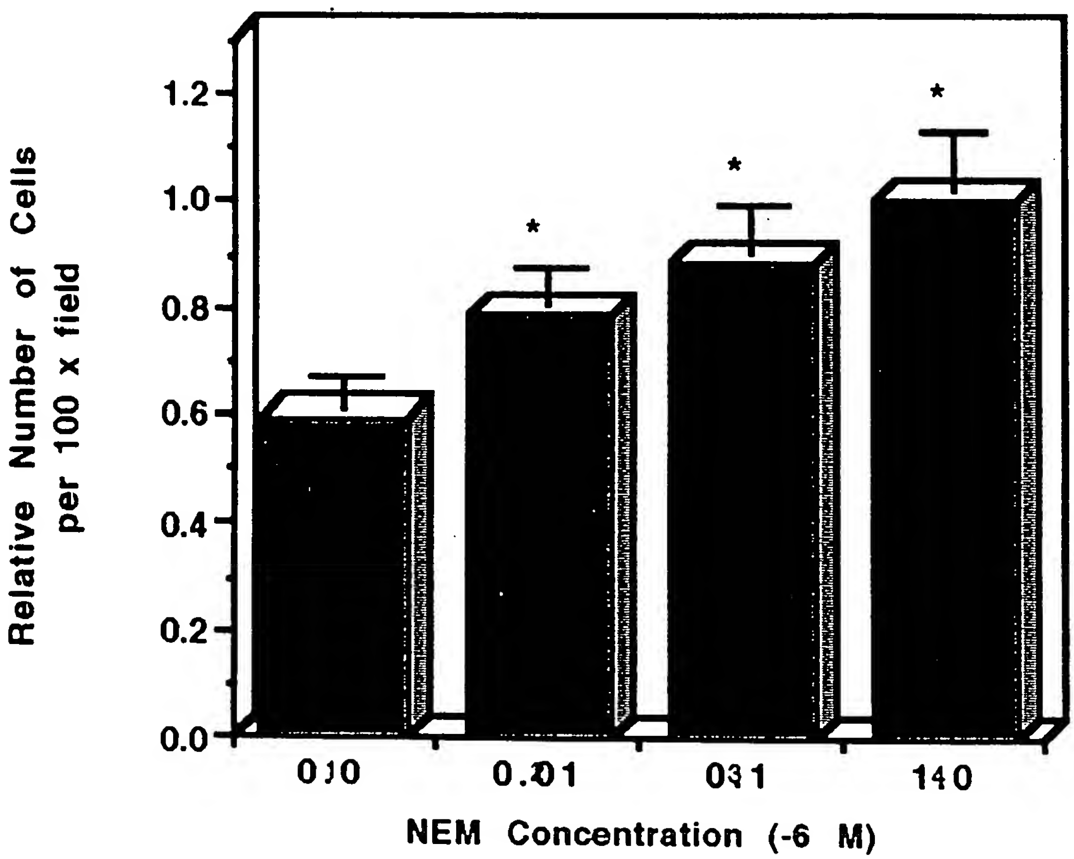

Relative Number of Cells
per 100 x field
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
*
*
NEM Concentration (-6 M)

FIG. 2

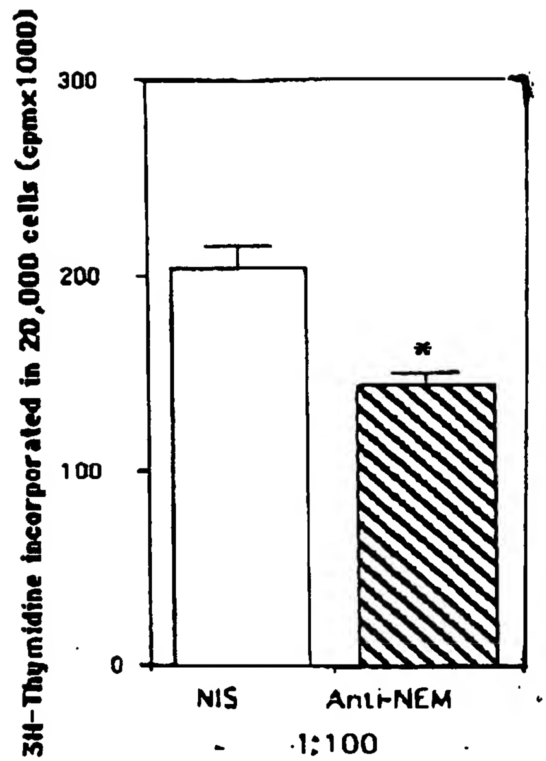

3H-Thymidine incorporated in 20,000 cells (cpmx1000)
300
200
100
0
*
NIS
Anti-NEM
1:100

FIG. 3

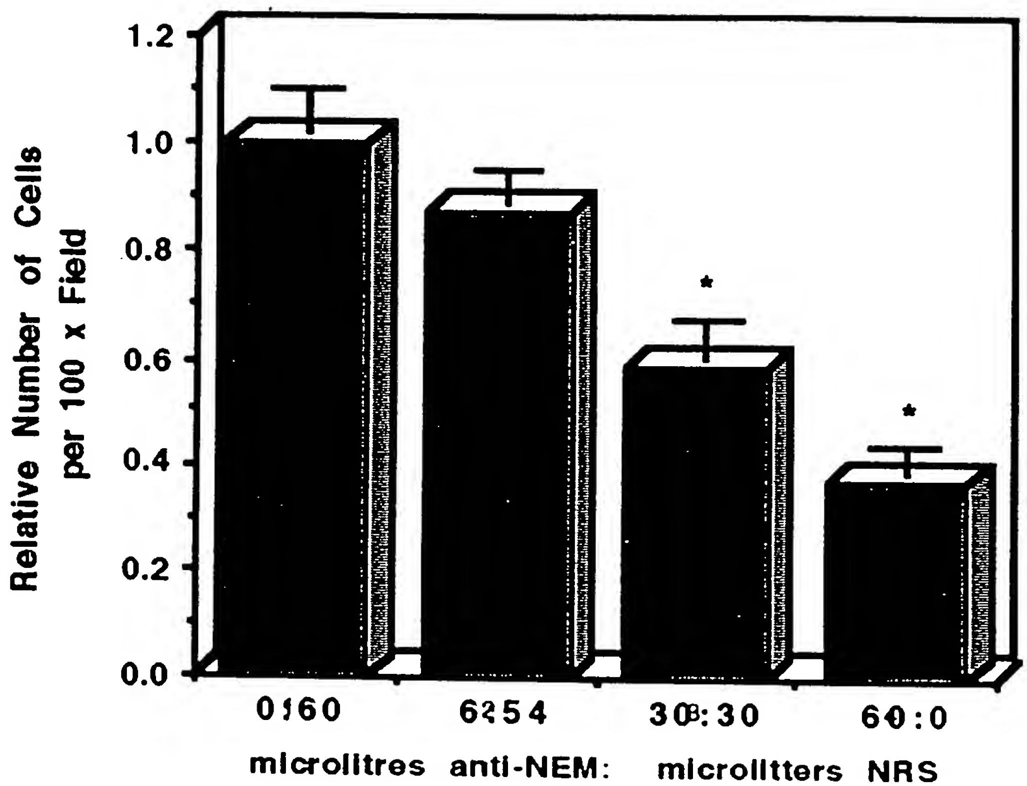
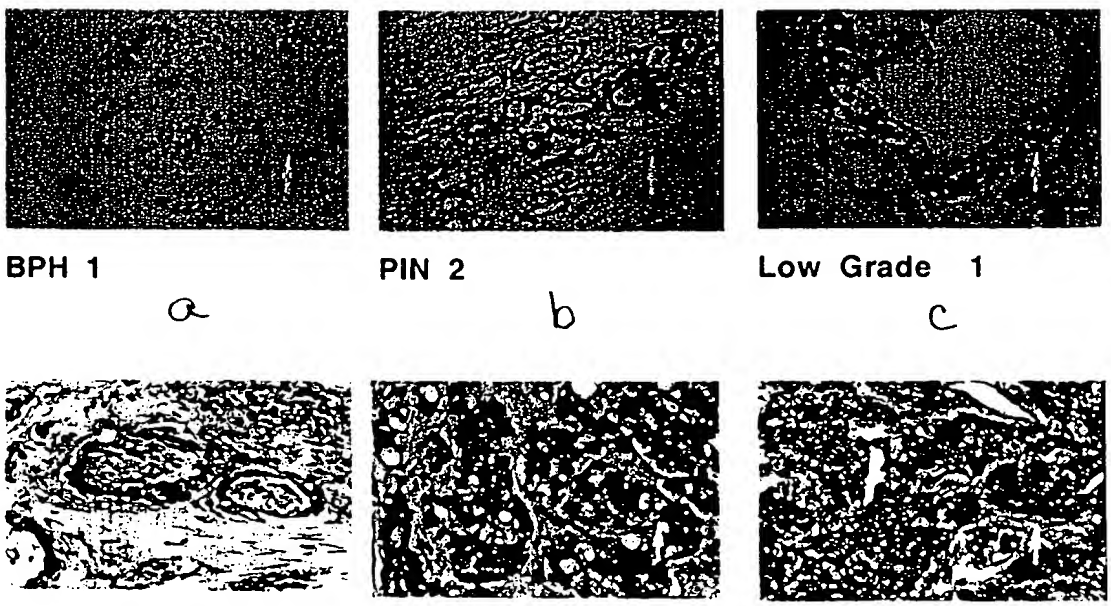

Relative Number of Cells per 100 x Field
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
*
0:60
6:54
30:30
60:0
microlitres anti-NEM: microlitters NRS

FIG. 4

PIN 1 Moderate Grade 1 High Grade 1

a b c

FIG. 5



BPH 1 a

PIN 2 b

Low Grade 1 c

Moderate Grade 2 d

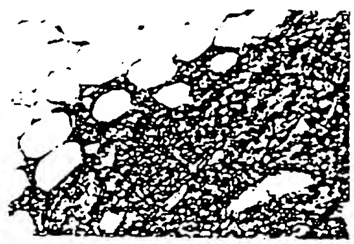
High Grade 2 e

Very Aggressive 1 f

FIG. 6

Liver Metastasis

a



Lymph Node Metastasis

b

Tonsils (negative control)

c

FIG. 7

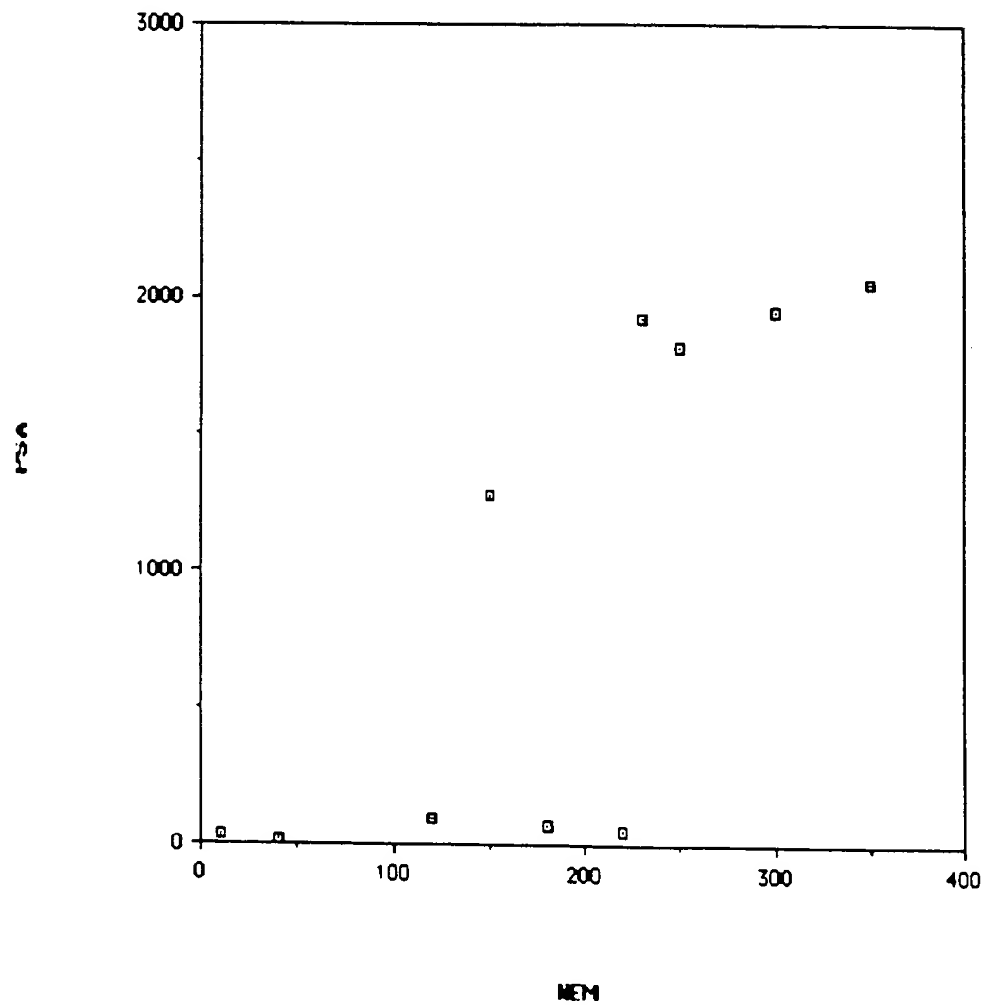


FIG. 8

NEM-INDUCED PROLIFERATION OF PROSTATE CANCER CELLS

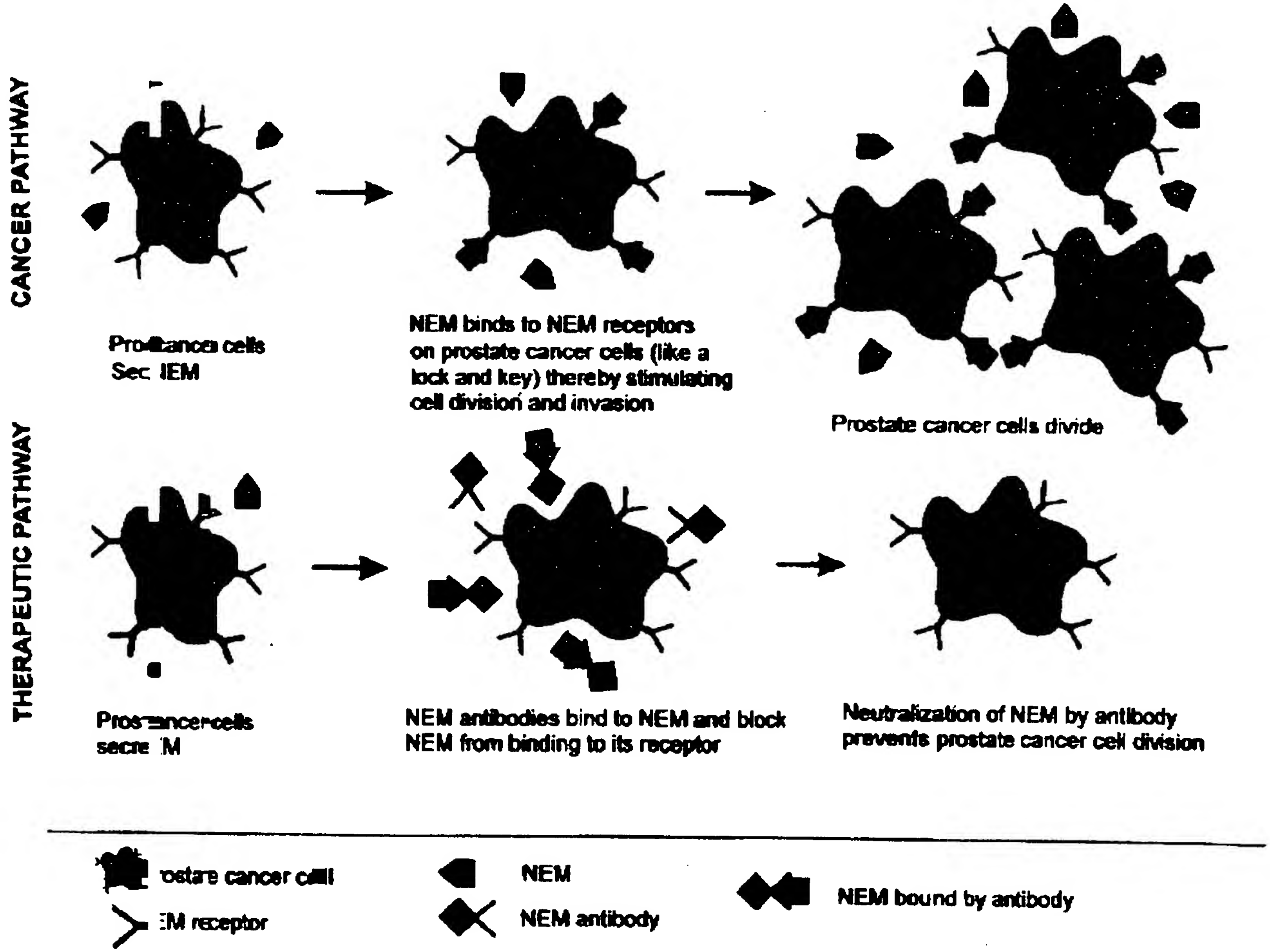


FIG. 9

Fig. 10 PROPOSED MECHANISM OF NEM-MEDIATED PROLIFERATION AND INVASION OF PROSTATE CANCER CELLS

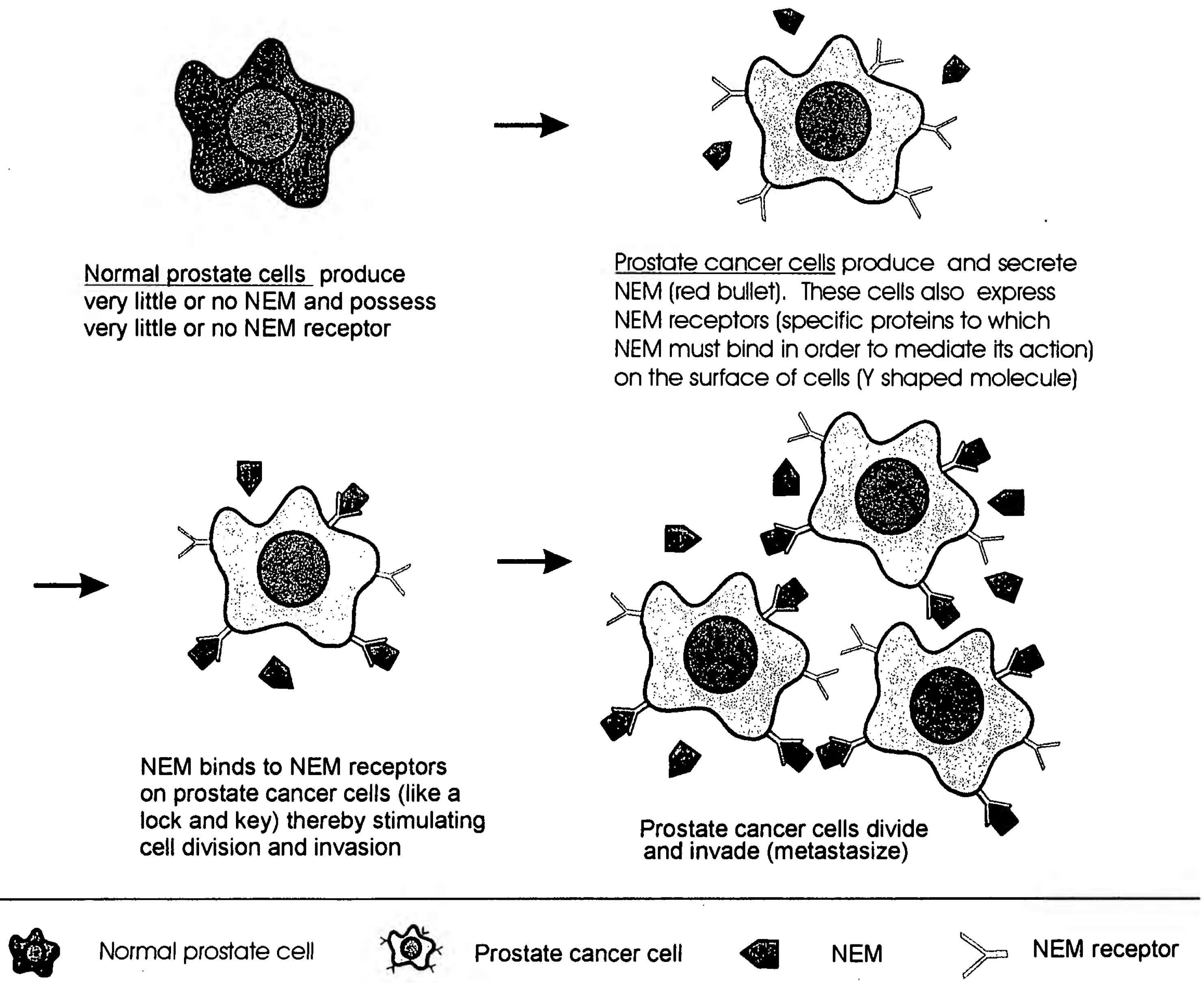


Fig.11: NEM-BASED CELL-TARGETED RADIATION THERAPY

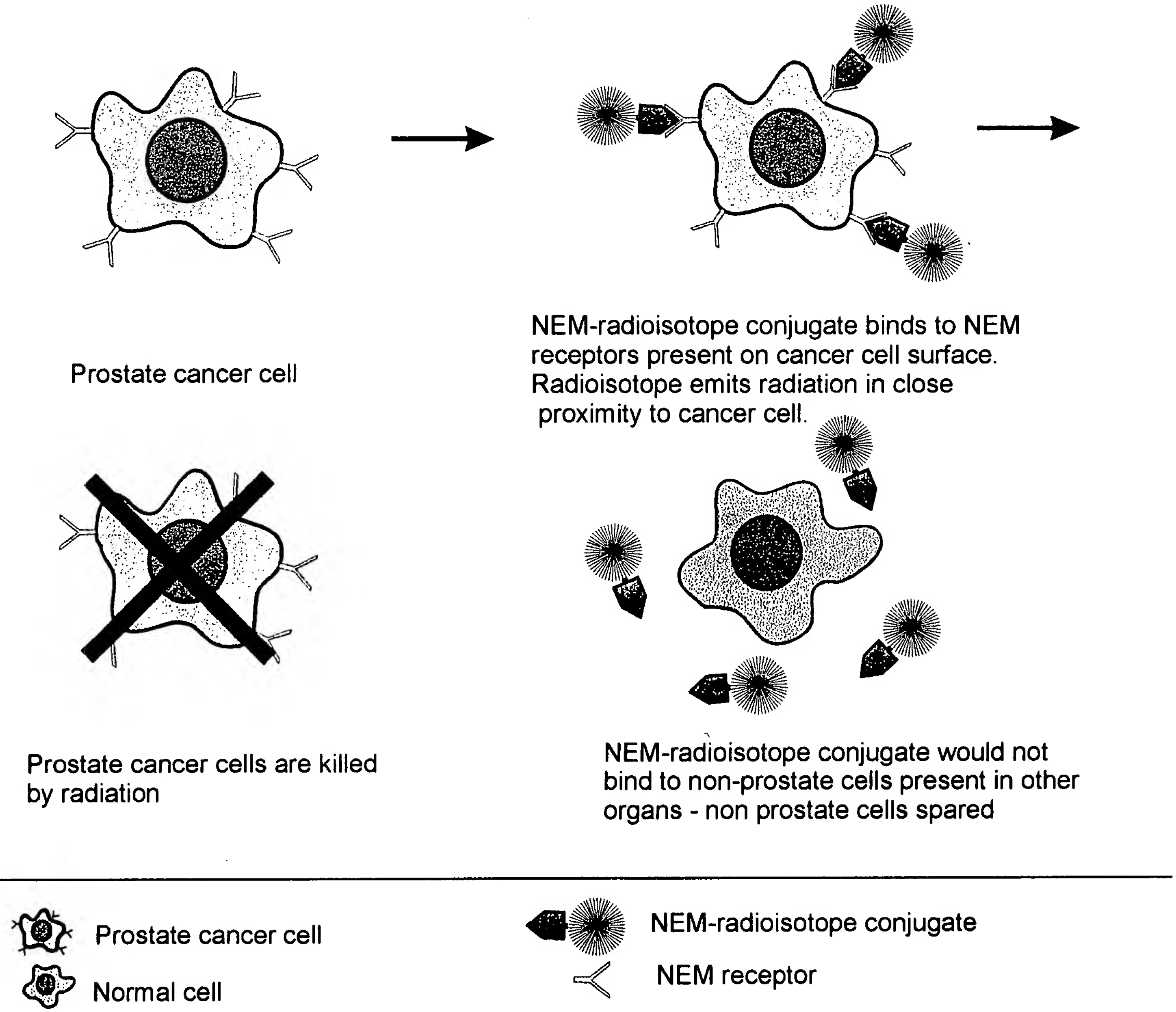


Fig.12: NEM ANTIBODY-BASED THERAPEUTIC FOR TREATMENT OF PROSTATE CANCER

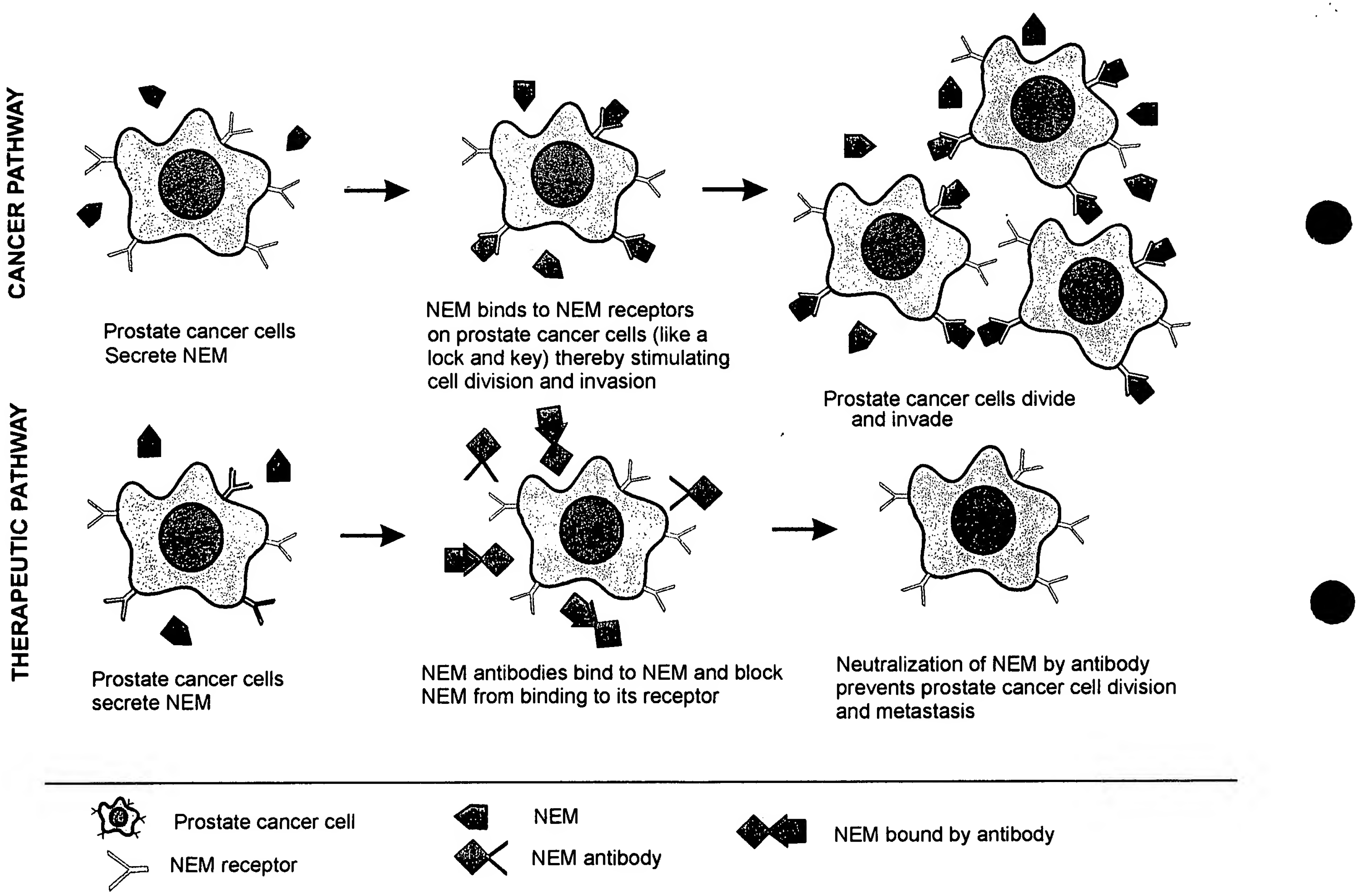


Fig. 13 NEM SELECTIVELY BINDS TO PROSTATE CANCER CELLS (Increased expression of NEM receptor in prostate cancer cells)

A section of a prostate cancer tissue showing NEM selectively binding to NEM receptor present on prostate cancer cells. Cells with NEM bound to them (cancer cells) are stained dark brown (wide band of cells on right abutting normal cells (pink) on the left. NEM is conjugated to a detection tag (digoxigenin- alkaline phosphatase) in order to visualize its binding to cancer cells. The data demonstrates the ability of NEM to direct itself to cancer cells and bind to them selectively. In the prostate cancer-imaging agent, one merely replaces the digoxigenin tag with a radioisotope like In-111. NEM would direct In-111 to prostate cancer cells wherever they are present in the body. The sites of accumulation of NEM-In-111 (sites of tumor) can be detected using a gamma camera. (The large dark blue spots are artifacts of the video camera).

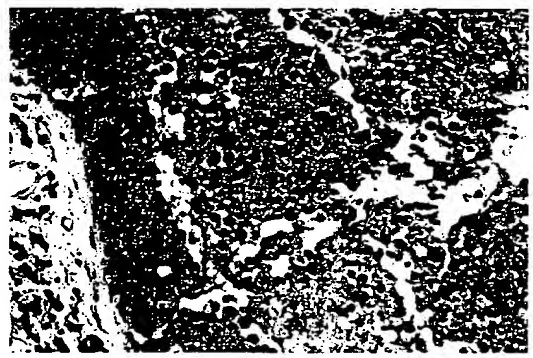
IMMUNOHISTOCHEMICAL DETECTION OF NEM IN PROSTATE CANCER TISSUE SECTIONS

Note: Areas stained blue are cells that produce NEM – cancer cells.

High-grade PIN, a precursor of invasive cancer.

Moderate-grade cancer.

High-grade cancer. Left portion is cancer tissue and the right portion is normal. Only the cancer tissue produces NEM (stained blue)

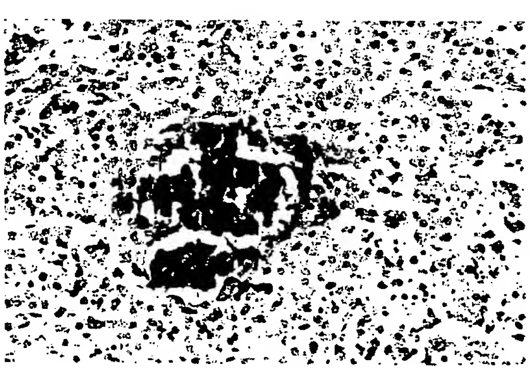


Aggressive cancer. The cancer cells (intense blue) grow haphazardly.

Immunohistochemisty stains for the presence of NEM peptide in cancer tissue. An antibody against NEM binds selectively to NEM, which is then detected by secondary reagents, which stains the tissue blue.

DETECTION OF NEM IN SECONDARY SITES OF PROSTATE CANCER

In situ hybridization



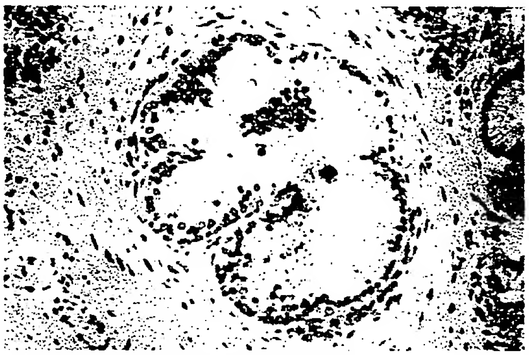
Section of a liver tissue showing a small prostate cancer secondary nodule. The cancer cells (in the middle) produce NEM (stained dark) while the surrounding liver tissue (stained pink) does not. This is a clear demonstration of the prostate cancer-specific nature of NEM

Fig. 14

*Fig.*15 EXPRESSION OF NEM IN BPH AND PROSTATE CANCER TISSUES SECTIONS

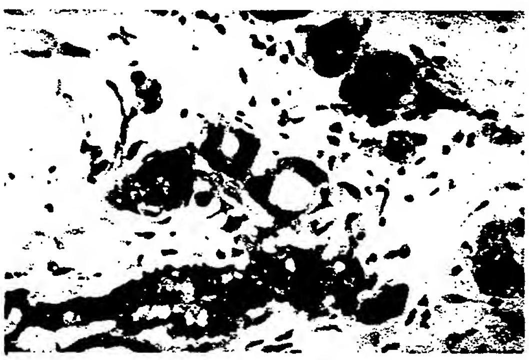
In situ hybridization.

Areas stained dark indicate cells producing NEM –note increased production of NEM with increasing grade of cancer – the more aggressive the cancer more the production of NEM. *High-grade PIN is a precursor of invasive cancer. BPH (benign prostatic hyperplasia) or prostate enlargement is a non-cancerous condition. Note loss of architecture of prostate tissue with advancing cancer. The cancer usually grows as small glands with the cancer cells lining the glands (see moderate grade cancer). In high-grade cancer, the glandular shape is lost and the cells grow in a haphazard fashion.*

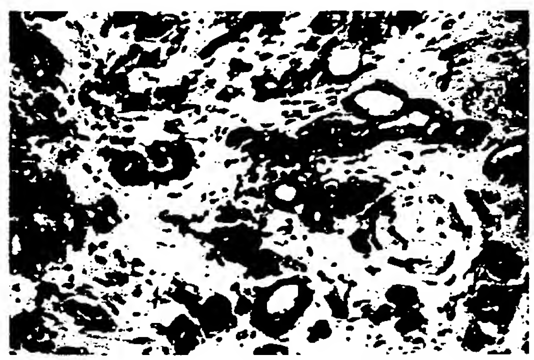


BPH (enlarged prostate)
No expression of NEM

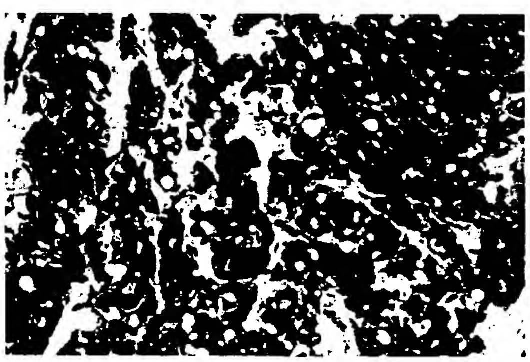
High-grade PIN (pre-cancerous lesion). Some moderate-grade cancer can also be seen



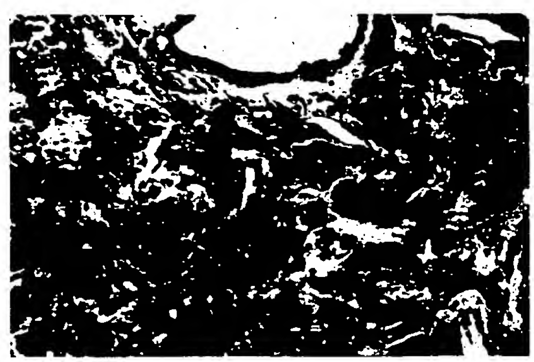
Moderate-grade cancer . Cancer cells line the small glands. Higher magnification



Moderate-grade cancer (note large number of small glands)



High-grade cancer



Aggressive cancer

The levels of NEM expression in prostate tissue is estimated by determining the level of NEM mRNA using NEM-specific probes and reagents, which stains cells expressing NEM.

Fig. 15

DNASIS ***** DNA TRANSLATION LIST ***** DATE 02-13-99

SEQUENCE 3 : 1-433

*** DNA TRANSLATION ***

```
                 10        20        30        40        50        60
          5'AGAACCTGTGTGCTGGGCTACCTGCATATAGTGCCAGAGTTCATCGAATCTCAGCTGCTG
SEQ 6 ---->  R  T  C  V  L  G  Y  L  H  I  V  P  E  F  I  E  S  Q  L  L
SEQ 7 ---->   E  P  V  C  W  A  T  C  I  *  C  Q  S  S  S  N  L  S  C  W
SEQ 8 ---->    N  L  C  A  G  L  P  A  Y  S  A  R  V  H  R  I  S  A  A  G

                 70        80        90       100       110       120
            GGGCTCCTTAGTCCTGTTTCACTTTAACCATATGCAAGACATTCCTCAACGTTATAGGCA
            G  L  L  S  P  V  S  L  *  P  Y  A  R  H  S  S  T  L  *  A
             G  S  L  V  L  F  H  F  N  H  M  Q  D  I  P  Q  R  Y  R  Q
              A  P  *  S  C  F  T  L  T  I  C  K  T  F  L  N  V  I  G  K

                130       140       150       160       170       180
            AGTAGACTGCATCTTTTTTTTTCTTTTTTTTTCTTTTTCTTTTTTCTTTTTTTCGGAGCT
            S  R  L  H  L  F  F  S  F  F  F  F  F  F  F  L  F  F  G  A
             V  D  C  I  F  F  F  L  F  F  S  F  S  F  F  F  F  S  E  L
              *  T  A  S  F  F  F  F  F  F  L  F  L  F  S  F  F  R  S  W

                190       200       210       220       230       240
            GGGGACCGAACCCAGGACCTTGCGCTTGCTAGGCAAGCGCTCTACCACTGAGCTAAATCC
            G  D  R  T  Q  D  L  A  L  A  R  Q  A  L  Y  H  *  A  K  S
             G  T  E  P  R  T  L  R  L  L  G  K  R  S  T  T  E  L  N  P
              G  P  N  P  G  P  C  A  C  *  A  S  A  L  P  L  S  *  I  P

                250       260       270       280       290       300
            CCAACCCCGACTGCATCGTTTTTGGTTTTTAGTTAAATTCCGGTTTGCTCTATTTCGTGT
            P  T  P  T  A  S  F  L  V  F  S  *  I  P  V  C  S  I  S  C
             Q  P  R  L  H  R  F  W  F  L  V  K  F  R  F  A  L  F  R  V
              N  P  D  C  I  V  F  G  F  *  L  N  S  G  L  L  Y  F  V  F

                310       320       330       340       350       360
            TCCCTTTGTTTAAAAGAAACTGTAGCCGGGGTAGTATATGTCTATAATCCCAGCAGTTGG
            S  L  C  L  K  E  T  V  A  G  V  V  Y  V  Y  N  P  S  S  W
             P  F  V  *  K  K  L  *  P  G  *  Y  M  S  I  I  P  A  V  G
              P  L  F  K  R  N  C  S  R  G  S  I  C  L  *  S  Q  Q  L  G

                370       380       390       400       410       420
            GAGGCAGGAGGATCCAGAGTTCAAGTCGGCATGGCACACATGAGACATTAGCTCAAAAAA
            E  A  G  G  S  R  V  Q  V  G  M  A  H  M  R  H  *  L  K  K
             R  Q  E  D  P  E  F  K  S  A  W  H  T  *  D  I  S  S  K  K
              G  R  R  I  Q  S  S  S  R  H  G  T  H  E  T  L  A  Q  K  K

                430
            AAAAAAAAAAAAA 3'
            K  K  K  K
             K  K  K  K
```

Fig. 16

DNASIS ***** DNA TRANSLATION LIST ***** DATE 02-13-99

SEQUENCE 3 : 1-433

*** DNA TRANSLATION ***

5'AGAACCTGTGTGCTGGGCTACCTGCATATAGTGCCAGAGTTCATCGAATCTCAGCTGCTG
GGGCTCCTTAGTCCTGTTTCACTTTAACCATATGCAAGACATTCCTCAACGTTATAGGCA
AGTAGACTGCATCTTTTTTTTTCTTTTTTTTTCTTTTTCTTTTTTCTTTTTTTCGGAGCT
GGGGACCGAACCCAGGACCTTGCGCTTGCTAGGCAAGCGCTCTACCACTGAGCTAAATCC
CCAACCCCGACTGCATCGTTTTTGGTTTTTAGTTAAATTCCGGTTTGCTCTATTTCGTGT
TCCCTTTGTTTAAAAGAAACTGTAGCCGGGGTAGTATATGTCTATAATCCCAGCAGTTGG
GAGGCAGGAGGATCCAGAGTTCAAGTCGGCATGGCACACATGAGACATTAGCTCAAAAAA
AAAAAAAAAAAAA 3'

Fig. 16 Con't

DNASIS ***** DNA TRANSLATION LIST ***** DATE 02-13-99

SEQUENCE 4 : 1-432

*** DNA TRANSLATION ***

```
                10        20        30        40        50        60
       5'AGAACCTGTGTGCTGGGCTACCTGCATATAGTGCCAGAGTTCATCGAATCTCAGCTGCTG
SEQ 9 →   R  T  C  V  L  G  Y  L  H  I  V  P  E  F  I  E  S  Q  L  L
SEQ 10 →   E  P  V  C  W  A  T  C  I  *  C  Q  S  S  S  N  L  S  C  W
SEQ 11 →    N  L  C  A  G  L  P  A  Y  S  A  R  V  H  R  I  S  A  A  G

                70        80        90       100       110       120
         GGGCTCCTTAGTCCTGTTTCCTTTAACCATATGCAAGACATTCCTCAACGTTATAGGCAA
          G  L  L  S  P  V  S  F  N  H  M  Q  D  I  P  Q  R  Y  R  Q
           G  S  L  V  L  F  P  L  T  I  C  K  T  F  L  N  V  I  G  K
            A  P  *  S  C  F  L  *  P  Y  A  R  H  S  S  T  L  *  A  S

               130       140       150       160       170       180
         GTAGACTGCATCTTTTTTTTTCTTTTTTTTTCTTTTTCTTTTTTCTTTTTTTCGGAGCTG
          V  D  C  I  F  F  F  L  F  F  S  F  S  F  F  F  F  S  E  L
           *  T  A  S  F  F  F  F  F  F  L  F  L  F  S  F  F  R  S  W
            R  L  H  L  F  F  S  F  F  F  F  F  F  F  L  F  F  G  A  G

               190       200       210       220       230       240
         GGGACCGAACCCAGGACCTTGCGCTTGCTAGGCAAGCGCTCTACCACTGAGCTAAATCCC
          G  T  E  P  R  T  L  R  L  L  G  K  R  S  T  T  E  L  N  P
           G  P  N  P  G  P  C  A  C  *  A  S  A  L  P  L  S  *  I  P
            D  R  T  Q  D  L  A  L  A  R  Q  A  L  Y  H  *  A  K  S  P

               250       260       270       280       290       300
         CAACCCCGACTGCATCGTTTTTGGTTTTTAGTTAAATTCCGGTTTGCTCTATTTCGTGTT
          Q  P  R  L  H  R  F  W  F  L  V  K  F  R  F  A  L  F  R  V
           N  P  D  C  I  V  F  G  F  *  L  N  S  G  L  L  Y  F  V  F
            T  P  T  A  S  F  L  V  F  S  *  I  P  V  C  S  I  S  C  S

               310       320       330       340       350       360
         CCCTTTGTTTAAAAGAAACTGTAGCCGGGGTAGTATATGTCTATAATCCCAGCAGTTGGG
          P  F  V  *  K  K  L  *  P  G  *  Y  M  S  I  I  P  A  V  G
           P  L  F  K  R  N  C  S  R  G  S  I  C  L  *  S  Q  Q  L  G
            L  C  L  K  E  T  V  A  G  V  V  Y  V  Y  N  P  S  S  W  E

               370       380       390       400       410       420
         AGGCAGGAGGATCCAGAGTTCAAGTCGGCATGGCACACATGAGACATTAGCTCAAAAAAA
          R  Q  E  D  P  E  F  K  S  A  W  H  T  *  D  I  S  S  K  K
           G  R  R  I  Q  S  S  S  R  H  G  T  H  E  T  L  A  Q  K  K
            A  G  G  S  R  V  Q  V  G  M  A  H  M  R  H  *  L  K  K  K

               430
         AAAAAAAAAAAA 3'
          K  K  K  K
           K  K  K
```

Fig. 17

SEQUENCE 4 : 1-432

5'AGAACCTGTGTGCTGGGCTACCTGCATATAGTGCCAGAGTTCATCGAATCTCAGCTGCTG
GGGCTCCTTAGTCCTGTTTCCTTTAACCATATGCAAGACATTCCTCAACGTTATAGGCAA
GTAGACTGCATCTTTTTTTTTCTTTTTTTTTCTTTTTCTTTTTTCTTTTTTTCGGAGCTG
GGGACCGAACCCAGGACCTTGCGCTTGCTAGGCAAGCGCTCTACCACTGAGCTAAATCCC
CAACCCCGACTGCATCGTTTTTGGTTTTTAGTTAAATTCCGGTTTGCTCTATTTCGTGTT
CCCTTTGTTTAAAAGAAACTGTAGCCGGGGTAGTATATGTCTATAATCCCAGCAGTTGGG
AGGCAGGAGGATCCAGAGTTCAAGTCGGCATGGCACACATGAGACATTAGCTCAAAAAAA
AAAAAAAAAAAA 3'

FIG. 17 Con't

Sequence 5

NEM DNA SEQUENCE 1-435

ATTAGAACCT GTGTGCTGGG CTACCTGCAT ATAGTGCCAG AGTTCATCGA ATCTCAGCTG CTGGGGCTCC

TTAGTCCTGT TTCCTTTAAC CATATGCAAG ACATTCCTCA ACGTTATAGG CAAGTAGACT GCATCTTTTT

TTTTCTTTTT TTTTCTTTTT CTTTTTTCTT TTTTTCGGAG CTGGGGACCG AACCCAGGAC CTTGCGCTTG

CTAGGCAAGC GCTCTACCAC TGAGCTAAAT CCCCAACCCC GACTGCATCG TTTTTGGTTT TTAGTTAAAT

TCCGGTTTGC TCTATTTCGT GTTCCCTTTG TTTAAAAGAA ACTGTAGCCG GGGTAGTATA TGTCTATAAT

CCCAGCAGTT GGGAGGCAGG AGGATCCAGA GTTCAAGTCG GCATGGCACA CATGAGACAT TAGCTCAAAA

AAAAAAAAAA AAAAA

FIG. 18

NEM PEPTIDE SEQUENCE 12

Ile Arg Thr Cys Val Leu Gly Tyr Leu His Ile Val Pro Glu Phe Ile Glu Ser

Gln Leu Leu Gly Leu Leu Ser Pro Val Ser Leu

FIG. 19